**National Toxicology Program**
Toxicity Report Series
Number 19

# NTP Technical Report on Toxicity Studies of

# Formic Acid

**(CAS No: 64-18-6)**

## Administered by Inhalation to F344/N Rats and B6C3F$_1$ Mice

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The NTP report on the toxicity studies of formic acid is based primarily on the 2-week studies that began in August, 1987, and ended in September, 1987, and the 13-week studies that began in December, 1987, and ended in March, 1988, at Battelle Northwest Laboratories.

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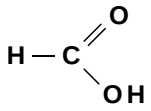

**Molecular Formula:** HCOOH
**CAS No.:** 64-18-6
**Molecular Weight:** 46

**Synonyms:** Aminic Acid, Formylic Acid, Methanoic Acid, Hydrogen Carboxylic Acid

# ABSTRACT

Formic acid occurs in a variety of plants and fruits, mammalian tissues, and insect venoms. It is used industrially in preparing a variety of drugs, dyes, and chemicals; as a decalcifier; and in leather tanning. Formic acid also is an environmental contaminant of air and water and has been identified as the toxic intermediate (formate) in methanol poisoning. Two- and 13-week toxicity studies of formic acid were conducted in male and female F344/N rats and B6C3$F_1$ mice by whole body inhalation exposure to formic acid vapors. In addition, *in vitro* genetic toxicity studies were performed with *Salmonella typhimurium,* with or without metabolic activation. Formic acid was not mutagenic in this assay.

In 2-week studies, groups of 5 F344/N rats and 5 B6C3$F_1$ mice of each sex were exposed to formic acid for 6 hours a day, 5 days a week, at concentrations of 0, 31, 62.5, 125, 250, or 500 ppm. Deaths occurred in animals exposed to 500 ppm (rats and mice) and 250 ppm (1 female mouse). Microscopic lesions in the respiratory and olfactory epithelia occurred in rats and mice exposed to 62.5 ppm and higher concentrations, with the severity related to the exposure concentration. The lesions consisted of squamous metaplasia, necrosis, and inflammation. Exposures had minimal or no effects on coagulation times, blood pH and electrolytes, or on concentrations and activities of urine analytes in rats during the 2-week studies.

In 13-week studies, groups of 10 animals of each species and sex were exposed to formic acid at concentrations of 0, 8, 16, 32, 64, and 128 ppm for 6 hours a day, 5 days a week. Two mice, 1 male and 1 female, died in the 128 ppm groups. Body weight gains were significantly decreased in mice exposed to 64 and 128 ppm formic acid. Microscopic changes in rats and mice ranged from minimal to mild in severity and generally were limited to animals in the 128 ppm groups. Lesions related to exposure to formic acid consisted of squamous metaplasia and degeneration

of the respiratory and olfactory epithelia, respectively. Hematologic and serum biochemical changes at interim and terminal time points were minimal to mild and, generally, were consistent with hemoconcentration.

Overall, the effects of formic acid were consistent with those of irritant chemicals administered by inhalation exposure. The no-observed-adverse-effect level (NOAEL) for respiratory injury was 32 ppm in rats and mice. There was no significant evidence of systemic toxicity in these studies.

# PEER REVIEW

## Peer Review Panel

The members of the Peer Review Panel who evaluated the draft report on the toxicity studies on formic acid on July 10, 1991, are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, panel members act to determine if the design and conditions of the NTP studies were appropriate and to ensure that the toxicity study report fully and clearly presents the experimental results and conclusions.

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## Summary of Peer Review Comments

On July 9 and 10, 1991, the Technical Reports Review Subcommittee of the Board of Scientific Counselors for the National Toxicology Program met in Research Triangle Park, NC, to review the draft technical report on toxicity studies of formic acid

Dr. M. Thompson, NIEHS, introduced the short-term toxicity study report by reviewing the natural occurrences and uses of formic acid, the experimental design, and the results.

Dr. Carlson, a principal reviewer, said the study was well done. He asked at what point after the 2-week study the blood pH was determined, noting that adidosis is an important problem with the acute toxicity of methanol through its metabolism to formate. Dr. Thompson said pH was determined the day following the last exposure to formic acid. Dr. Carlson also asked that a rationale be given for administering the chemical by inhalation. Dr. Thompson said that formic acid had been nominated for study because of its structural relationship with formaldehyde and because inhalation is an important route of exposure for humans.

Dr. Klaassen, a second principal reviewer, said the study was performed well. He said he was concerned that the report may over-emphasize that rodent data on formic acid exposure may not be applicable to humans. He said the localized toxic effects observed might be very relevant for humans. Dr. Thompson said the lack of a systemic toxic effect in rats may be related to their resistance to formate toxicity, and that this was the reason for the emphasis. Dr. Klaassen agreed but said that the possible similarity in local toxic effects among rodents and humans should be made more clear.

Dr. Zeise questioned the NOAEL reported in the study (32 ppm), noting a reported olfactory epithelial lesion in a male rat at 32 ppm in the 13-week study. Dr. M. Elwell, NIEHS, said the olfactory degeneration was a minimal change and that it was difficult to cite it as a treatment effect.

After discussion of editorial matters, the panel agreed to accept to report, with the suggested changes.

# INTRODUCTION

## Physical Properties, Occurrence, Production, Uses, and Exposure

Formic acid, a colorless, highly caustic liquid with a pungent odor, has a melting point of 8.4°C, a boiling point of 100.7°C, a density of 1.220 g/cm$^3$ at 20°C, and a vapor pressure of 400 mm Hg at 24°C. Vapor and liquid forms of formic acid are flammable, especially at temperatures greater than 69°C. Formic acid is miscible with water, alcohol, and ether, soluble in benzene and toluene, and very soluble in acetone. It can react as an acid and as an aldehyde (Wagner, 1980). Under normal storage conditions formic acid can deteriorate by dehydration, dehydrogenation, or through a bimolecular redox reaction.

Formic acid, first described by Fisher in 1670 in the products resulting from the distillation of red ants (Windholz, 1983), occurs in both natural and man-made sources in the environment. A constituent of ant, wasp, and bee venom, formic acid also occurs in mammalian muscle tissue, sweat, and urine. It is found in plants, such as in the needles of the Douglas fir, and in unripened grapes, peaches, raspberries, strawberries, petitgrain lemon, and in bitter orange (SRI, 1981). It also is present in many foods (Gley, 1967), e.g., fruits (20 - 40 ppm), fruit juices (30 - 100 ppm), fruit syrups (650 - 1630 ppm), honey (20 - 2000 ppm), wines (1 - 340 ppm), coffee, roasted (1350 - 2200 ppm), coffee, extracts (2000 - 7700 ppm), evaporated milk (30 - 400 ppm), and cheese (20 - 200 ppm) (Tracor Jitco, 1974).

An air and water pollutant, formic acid has been measured at concentrations ranging from 4 to 72 ppm in the atmosphere. It has been identified in river and surface water, in unfinished industrial waste water, and in municipal sewage and final municipal discharge water at concentrations ranging from approximately 10 to 80,000 µg/L (SRI, 1981). Other sources of formic acid include forest fires, lacquer manufacturing, trash and plastic burning, and tobacco smoke. Thermal degradation of polyethylene during manufacturing may result in the release of formic acid and various aldehydes into the atmosphere (Zitting and Savolainen, 1980).

Formic acid is synthesized industrially by heating carbon monoxide and sodium hydroxide under pressure, then treating the resulting sodium formate with sulfuric acid. It also is prepared by acid hydrolysis of methyl formate and as a by-product in the manufacture of acetaldehyde and formaldehyde (Wagner, 1980). U.S. production and import figures for formic acid between 1976 and 1979 ranged from 2.3 to 3.1 x $10^7$ kg/year to 3.7 x $10^3$ to 1.0 x $10^6$ kg/year, respectively.

Formic acid is used extensively as a decalcifier, as an acidulating agent in textile dying and finishing, and in leather tanning (Wagner, 1980). It also is used in the preparation of organic esters and in the manufacture of drugs, dyes, insecticides, and refrigerants. Other functions include use as a mold inhibitor on grain and silage, as a solvent for perfumes, as a plasticizer for vinyl resin, and as a coagulator for latex. Trace amounts of formate esters are used in the formulation of fragrances and flavors, especially fruit and honey mixtures (Wagner, 1980; Sittig, 1985).

The Threshold Limit Value (TWA) for exposure to airborne formic acid is 5 ppm or 9 mg/m$^3$ and is based on irritation to the respiratory tract (ACGIH, 1986). No criteria for a permissible ambient water standard have been set; however, the EPA has suggested a level of 124 mg/L (Sittig, 1985).

According to NIOSH (1980), approximately 533,799 workers were exposed occupationally to formic acid during the period 1972 to 1974. Public exposure results from the consumption of various food products such as fruit juices, honey, wines, coffee, unripened grapes, and strawberries. Human exposure also occurs through exposure to the atmosphere and water because of the compound's presence in the environment.

## Human Toxicity

Formic acid is caustic and can cause damage to skin, eyes, and mucosal surfaces (International Labour Office, 1983). Chronic absorption has been reported to cause albuminuria and hematuria (Windholz, 1983). Inhalation of formic acid results in rhinitis, cough, bronchitis, and dyspnea; ingestion causes corrosion and necrosis of the mucous membranes of the mouth, throat, esophagus, and stomach. Extensive exposure can produce depression of the central nervous system, severe metabolic acidosis, and nephropathy (Seiler *et al.,* 1988). Swallowing formic acid has resulted in a number of cases of severe poisoning and death (Sittig, 1985). In some cases of fatal poisoning, hematuria and anuria develop, and the patient may die from uremia, circulatory failure, or pneumonia. Air levels of formic acid were measured at 15 ppm in a textile plant in which workers were complaining of nausea (ACGIH, 1986).

## Animal Studies

Oral $LD_{50}$ values for formic acid in rats range from 1100 to 1850 mg/kg. $LD_{50}$ values for mice range from 700 to 1100 mg/kg for oral, 940 mg/kg for i.p., and 145 mg/kg for i.v. administration. Reported $LD_{50}$ values from inhalation studies were 15 g/m$^3$/15 minutes for rats and 6.2 g/m$^3$/15 minutes for mice. Clinical signs included respiratory distress and unidentified behavioral changes. An oral $LD_{50}$ value of 4000 mg/kg has been reported for dogs (Malorny, 1969; NIOSH, 1985; Sax and Lewis, 1989).

Reductions in body weight gains at the higher doses were the only effects seen in studies in which young rats (~40 g, strain unspecified) were administered formic acid in the diet or drinking water at levels of 0.5 or 1.0% for 6 weeks, or in which rats received 8 to 360 mg/kg formic acid in drinking water for 2 to 27 weeks (Clayton and Clayton, 1981). Hypochromic anemia and a mild lymphocytosis developed in rats receiving formic acid in the diet. It has been reported that the survival of offspring obtained from female rats administered 1.0% formic acid in drinking water for up to 7 months was reduced by 50 to 67% (Tracor Jitco, 1974). No other references to studies of reproductive toxicity or teratogenicity of formic acid were located.

There were small but statistically significant changes in activities of drug metabolizing as well as in other enzymes in liver, kidney, and brain of male Wistar rats exposed to 20 ppm formic acid vapor for 3 or 8 days, 6 hours per day (Zitting and Savolainen, 1980). Concentrations of glutathione in brain, liver, and kidney were decreased in exposed rats; activities of lysosomal

acid proteinase in brain and ethoxycoumarin deethylase in liver were increased, while those of cytochrome P-450 in kidney were decreased compared to controls.

## Disposition and Metabolism

Formic acid is absorbed from the gastrointestinal tract, lungs, intact skin, and urinary bladder. The absorbed compound is oxidized to $CO_2$ and $H_2O$, partly excreted unchanged in the urine, and partly metabolized in tissues. The main site of oxidation is the liver, although intestinal mucosa, lungs, kidney, and spleen also contribute. Oxidation of formate occurs by folate-dependent and catalase-peroxidative mechanisms. In rats, monkeys, and human beings, half-lives of sodium formate in blood are 12 - 23, 31 - 51, and 55 minutes, respectively (Clay *et al.*, 1975; McMartin *et al.,* 1977; Rietbrock *et al.,* 1971). The rate of formate oxidation to $CO_2$ in monkeys was markedly lower than that in rats. Although the rate of oxidation was dose-dependent in both species, metabolism in monkeys proceded at a rate approximately one-half that measured in rats (McMartin *et al.*, 1977). Rates of formate oxidation were 40 mg/kg/hr in monkeys, 300 mg/kg/hr in mice, and 78 mg/kg/hr in rats. Excretion of formic acid also is influenced by the amount administered; 8% - 9% was excreted unchanged by dogs given a 1 g oral dose as compared to 65% excreted by dogs given a 5 g dose (Tracor Jitco, 1974).

Methyl chloride and formaldehyde are metabolized to formate, which is metabolized further by folic acid-dependent pathways; then they either are incorporated into tissue macromolecules or oxidized to $CO_2$ and $H_2O$ (Kornbrust *et al.,* 1982; Mashford *et al.,* 1982). In addition, methanol toxicity is associated with accumulation of formate (McMartin *et al.,* 1977; Clay *et al.,* 1975; Martin-Amat *et al.,* 1978). Methanol is rapidly metabolized to formaldehyde primarily by the catalase-peroxidative system in rats, although the alcohol dehydrogenase and microsomal cytochrome P-450 enzyme systems are active in the rat, guinea pig, and rabbit (Tephly *et al.,* 1964; Mannering *et al.,* 1969; Teschke *et al.,* 1975; Dalvi and Townsend, 1976). Only human beings and monkeys rely primarily on the alcohol dehydrogenase system. Formaldehyde is rapidly metabolized to formate by formaldehyde dehydrogenase (Uotila and Koivusalo, 1974a; 1974b) and does not accumulate in rats or monkeys after dosing with methanol (Makar and Tephly, 1977; McMartin *et al.,* 1977, 1979). Formaldehyde metabolism to formate also can occur by various aldehyde dehydrogenases. In primates and rodents, oxidation of formate to $CO_2$ is accomplished primarily by folate-dependent metabolism (McMartin *et al.,* 1979; Palese and Tephly, 1975). Although of lesser importance, this step also can be catalyzed by catalase peroxidative oxidation in rodents. Urinary excretion of formate may be an important route of elimination in folate-deficient rodents (Smith and Taylor, 1982).

The susceptibility of a species to methanol toxicity is inversely related to its capacity for tetrahydrofolate-dependent oxidation of formate (McMartin *et al.,* 1977). Tetrahydrofolate levels in the liver of monkeys are 60% of those in rats and are thought to account for a 50% lower maximal rate of formate oxidation in monkeys as compared to rats (Black *et al.,* 1985). Inhibition of methionine synthetase, an enzyme important in the synthesis of tetrahydrofolate, by administering nitrous oxide ($N_2O$) or feeding a folate-deficient diet, renders rats susceptible to methanol toxicity (Eells *et al.,* 1981; Makar and Tephly, 1977). In a recent study, activities of the enzyme, 10-formyl-tetrahydrofolate dehydrogenase, which catalyzes the oxidation of 10-formyl-tetrahydrofolate to $CO_2$ and tetrahydrofolate, were compared in human and rat liver (Johlin *et al.,*

1989). The finding that properties of the enzymes were similar, but that the activity was lower in human than in rat liver, may be an additional factor contributing to the accumulation of high levels of formate during methanol metabolism in humans as compared to rats. On a quantitative basis, this indicates that humans should be more susceptible to formate toxicity than the rat.

## Chronic Toxicity/Carcinogenicity

Formic acid and several other chemicals were tested for tumor promotion in a dermal exposure study. An 8% formic acid and water solution was applied on both sides of both ears of male Swiss mice (Frei and Stephens, 1968). The animals' ears were pretreated with one application of 1.5% 7, 12-dimethylbenz(a)anthracene (DMBA). The formic acid solution was applied to the ears twice a week for 20 weeks with the first application occurring 1 week after treatment with DMBA. Animals treated with formic acid had hyperplasia and epidermal thickening at incidences similar to or below those of controls; formic acid was concluded not to be a tumor promoter in this study.

## Genetic Toxicity

Formic acid (10 - 3333 µg/plate) was not mutagenic in *Salmonella typhimurium* strains TA100, TA1535, TA97, or TA98, with or without S9 (Zeiger *et al.,* 1992). It also did not induce sister-chromatid exchanges, with or without S9, in hamster V79 cells treated with a maximum dose of 2mM (Basler *et al.,* 1985).

Positive results were reported for formic acid in tests for induction of sex-linked recessive lethal mutations in germ cells of male Drosophila (Stumm-Tegethoff, 1969) and in tests for induction of chromosomal aberrations in Chinese hamster ovary cells (Morita *et al.,* 1990). However, in both these investigations, it was noted that neutralizing the acidic pH of the test solution or nutrient medium, or increasing the buffering power of the solutions, eliminated the mutagenic responses. It was concluded that formic acid by itself is non-mutagenic, but that testing at concentrations which produce non-physiological pH levels results in a "false positive" response due to perturbations in the test system.

## Study Rationale and Design

Formic acid was nominated as part of an air pollutant class study by the National Cancer Institute, based on its high potential for human exposure (~533,800 workers exposed occupationally from 1972 to 1974); its structural relationship to formaldehyde, a known nasal carcinogen in rats (Swenberg *et al.,* 1980); and because of the lack of information concerning the toxicity and carcinogenicity of the chemical. In response, the NTP conducted 2-week and 13-week inhalation studies using male and female B6C3F1 mice and Fischer 344/N rats. Mutagenicity studies were conducted in *Salmonella typhimurium,* using buffered solutions of formic acid to reduce the potential for false positive responses.

# MATERIALS AND METHODS

## Procurement and Characterization of Formic Acid

Formic acid was obtained from BASF Wyandotte Corporation (Parsippany, NJ). Cumulative analytical data for the chemical indicated a purity of approximately 95%, with approximately 5% water as the only significant contaminant. The infrared, ultraviolet/visible, and nuclear magnetic resonance spectra were consistent with the structure of formic acid and available literature references. Elemental analysis results for carbon and hydrogen agreed with theoretical values, corrected for the water content. Karl Fischer titrimetry indicated 4.87 ± 0.07% water. Functional group titration indicated a purity of 94.9 ± 0.3%. Gas chromatography by 1 system (10% SP-1000/1% $H_3PO_4$) resolved 1 peak. A second gas chromatography system (15% SP-1220/1% $H_3PO_4$) resolved a major peak and an impurity peak with an area of 2.0% relative to the area of the major peak. This impurity was identified tentatively as water, on the basis of retention time with water-spiked samples.

The chemical was administered to animals by inhalation exposure. Formaldehyde was determined as a possible degradation product of formic acid in the exposure chambers, and determinations of formaldehyde levels were made in occupied and unoccupied chambers containing 8 and 128 ppm formic acid as well as in the formic acid distribution line (~2500 ppm formic acid). Grab samples, using gas sampling tubes packed with N-benzylethanolamine coated on a solid support, were collected and subsequently analyzed by gas chromatography for the 3-benzyloxazolidine derivative. In addition, the stability of formic acid in the generator reservoir over extended time periods was investigated. These studies revealed no evidence of significant degradation of formic acid. The amount of formaldehyde collected was less than 0.1% of the collected amount of formic acid in all samples that were taken.

Repeated purity analyses of samples taken from the formic acid generator indicated that formic acid did not decompose in the generator reservoir over a period of at least 29 days. No significant discrepancies in formic acid purity were observed in any of these analyses.

## Vapor Generation

Animals were exposed and maintained in 1.7 $m^3$ inhalation chambers, commercially produced by Harford Systems, Inc., (Aberdeen, MD). Bulk liquid formic acid was contained in an 8-liter, stainless steel, nitrogen-blanketed reservoir confined within a vented steel cabinet. As the formic acid was used, nitrogen replaced the formic acid and served to exclude $O_2$ from the reservoir. Liquid to be vaporized was pumped from the reservoir to a vaporizer by a micrometering pump which was constructed of materials compatible with formic acid. All liquid delivery tubing was constructed of Teflon®.

The vaporizer consisted of a stainless steel cylinder covered with a glass fiber wick from which the liquid was vaporized. An 80-watt heater and 2 temperature sensing elements were incorporated within the cylinder. One sensing element was connected to a remote temperature

controller allowing vaporizer temperatures of up to 120°C, with control to better than ± 0.5°C. The vaporizer was operated at approximately 97 ± 5°C. The other sensing element was connected to a digital temperature readout device. Output from this device was recorded at periodic intervals. The cylindrical vaporizer was positioned in the fresh-air duct leading directly to the vapor distribution manifold.

After flash vaporization, the vapor entered a short distribution manifold where the individual delivery lines carried a metered amount of vapor to each exposure chamber. A constant concentration of formic acid vapor, approximately 2300 ppm, was maintained in the distribution duct. Dilution air was conditioned to room temperature at approximately 50% relative humidity and was filtered by HEPA and charcoal filters. Vacuum transvector pumps, located at the chamber end of each vapor delivery line, generated negative pressure to draw the formic acid vapor from the distribution manifold through fine metering valves to the chambers. The high-concentration vapor was diluted by conditioned air to achieve the required target concentration immediately before entry of the vapor into the chambers.

The time after the start of exposure for the concentration to reach 90% of the final stable concentration in the chamber (T90) and the time after the termination of generation for the vapor concentration to decay to 10% of the stable concentration (T10) were determined. T90, with and without animals in the chamber with a flow of 15 CFM, was 10 and 27 minutes, respectively. T10, with and without animals, was 10 - 11 minutes and 40 - 100 minutes, respectively.

## Concentration Monitoring

A Foxboro Miran 980 infrared spectrometer (The Foxboro Co., Foxboro, MA) with a 20-meter, variable-pathlength, heated (~80°C) gas cell was used to monitor the exposure chambers, control chamber, exposure room, an on-line standard of formic acid vapor, and a pure nitrogen source. All locations were monitored approximately once every 40 minutes. The infrared cell was set to a 9.75-meter pathlength; the analytical wavelength for formic acid was 9.050 microns. Water was measured at 6.535 microns to allow a small correction for the absorbance of water vapor at the analytical wavelength for formic acid. A reference measurement was performed at 4.045 microns to correct for instrument drift.

The on-line monitor was calibrated by comparing monitor readings with GC analyses of grab samples collected from the exposure chambers at the time of the readings. The limit of detection and limit of quantification for the on-line monitor were determined at an average chamber relative humidity of 33-51%. The practical detection limit was 0.36 ± 0.10 ppm, with a practical quantification limit of 0.68 ± 0.10 ppm. During the 2-week and the 13-week studies, at least 96% and 91%, respectively, of the measured concentrations for each chamber were within ± 10% of the target concentration.

## Toxicity Study Design

Male and female Fischer 344/N rats and B6C3F$_1$ mice used in these studies were produced under strict barrier conditions at Taconic Farms (Germantown, NY). Animals were the progeny of

defined microflora-associated parents that were transferred from isolators to barrier-maintained rooms. Rats and mice were shipped to the study laboratory at 4 weeks of age, quarantined at the study laboratory for 11-13 days, and placed on study at 6-7 weeks of age. Blood samples were collected and the sera analyzed for viral titers from 5 animals per sex and species at study start and at termination in the 13-week studies. Data from 5 viral screens performed in rats and 12 viral screens performed in mice (Boorman *et al.,* 1986; Rao *et al.,* 1989; 1989a) showed that there were no positive antibody titers.

During the acclimation period, animals were randomly assigned to test groups using body weight as the blocking variable. Once exposure began, the animals were housed continuously in exposure chambers with chamber doors closed, except during animal husbandry procedures. Pelleted NIH-07 feed (Zeigler Bros., Inc., Gardners, PA) was available to animals at all times except during the daily exposure period, when feed was removed. Drinking water was available *ad libitum*.

Groups of 5 rats and 5 mice of each sex were administered formic acid by inhalation exposure for 12 days, 6 hours per day +T90/day (30 minutes), 5 days per week. Exposure concentrations for rats and mice were 0, 31, 62.5, 125, 250, or 500 ppm. After the third day of exposure, rats were removed from the inhalation chambers and placed in metabolism cages for a 16-hour collection of urine. Animals had access to water but not food. Urine collection tubes were placed in ice/water baths. Measurements included volume, pH, concentrations of glucose and protein, and activities of aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), and alkaline phosphatase (AP). Rats were returned to the inhalation chamber after the collection period.

On the day following the end of the 2-week exposure period, blood samples were collected from rats for determination of pH, concentrations of serum electrolytes, and coagulation times. Immediately prior to termination, rats were anesthetized with an i.p. injection of sodium pentobarbital, and blood samples were collected into plastic syringes from the lumbar aorta. For measurement of blood pH, samples were quickly transferred to a capillary tube containing heparin, and a Radiometer BME-33 blood pH instrument (Radiometer America, Inc., Westlake, OH) was used for the analyses. For determination of concentrations of sodium, potassium, chloride, and total $CO_2$, blood samples were placed into tubes devoid of an anticoagulant, and serum was harvested. Instrumentation Laboratories instruments, Models 442 and 446 (Instrumentation Laboratories, Lexington, MA), were used for these determinations. For determination of prothrombin and partial thromboplastin times, samples were placed in 3.8% sodium citrate and tests were performed using Dade reagents and a BBL Fibrometer (BBL Microbiology Systems, Cockeysville, MD). Necropsy examinations were performed on all animals (rats and mice). Weights were determined for the liver, thymus, right kidney, right testis, heart, and lungs. The following tissues in all control and treated animals were trimmed, embedded, stained with H&E, and examined microscopically: gross lesions, larynx, lungs and attached tracheobronchial lymph nodes, nasal cavity, and trachea. Further details are outlined in Table 1.

In the 13-week studies, rats and mice in groups of 10 per sex were exposed to formic acid vapor by whole body exposure at target concentrations of 0, 8, 16, 32, 64, and 128 ppm for 6 hours +T90/day (30 minutes), 5 days/week. Ten additional male and female rats per group were

included for clinical pathology studies; clinical observations were recorded daily. Body weights were recorded at study start, at weekly intervals, and at the end of the studies. Organ weights were determined for the thymus, heart, right kidney, lungs, liver, and right testis.

Clinical pathology studies were performed on the additional rats on days 3 and 23, and on core study rats at study termination. Animals were anesthetized with 70% $CO_2$:30% $O_2$ and bled from the retroorbital sinus using heparinized microcapillary tubes. Samples for hematologic analyses (~0.50 mL) were collected in tubes containing dry potassium EDTA, gently mixed, and held at room temperature until analyzed. Blood samples for serum analyses (~0.75 mL) were collected into tubes containing a separator gel but without an anticoagulant. These samples were allowed to clot at room temperature for approximately 30 minutes and centrifuged at 5000 g for 10 minutes; the serum then was harvested for biochemical analyses.

Automated hematologic analyses were performed using an Ortho ELT-8/ds hematology system (Ortho Diagnostics Systems, Inc., Westwood, NJ). The following variables were measured (or calculated): erythrocyte, leukocyte, and platelet counts, hemoglobin (HGB) concentration, hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). Smears of peripheral blood were stained with Wrights stain and examined microscopically. Leukocyte differentials were determined on 100 cells; absolute counts of individual leukocytes were calculated based on the total leukocyte count and the relative number. Reticulocytes were stained by mixing equal volumes of whole blood with new methylene blue stain for 20 minutes. Relative numbers of reticulocytes, determined by microscopic examination of approximately 1000 erythrocytes, were converted to absolute counts based on the total erythrocyte count.

Biochemical analyses were performed using an Abbott VP analyzer (Abbott Laboratories, North Chicago, Ill.) The following assays were performed using reagents and methods provided by the manufacturer: urea nitrogen (UN), creatinine, total protein, albumin, alanine aminotransferase (ALT), alkaline phosphatase (AP), creatine kinase (CK), and amylase. For determination of activity of sorbitol dehydrogenase (SDH), a reagent kit was obtained from Sigma Chemical Company (St. Louis, MO) and adapted for the Abbott VP. For determination of total bile acids, a reagent kit was obtained from Nyegaard Diagnostica (Enzabile, Oslo, Norway), and concentrations were measured as an end-point reaction using a recording spectrophotometer.

A complete necropsy was performed on all animals. Organs and tissues were examined for gross lesions. Tissues were fixed in 10% neutral buffered formalin. Tissues for microscopic evaluation were dehydrated and embedded in paraffin, sectioned at approximately 5 microns, and stained with hematoxylin and eosin, and examined microscopically.

Upon completion of the histologic evaluation by the laboratory pathologist, the slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were sent to an independent pathology laboratory for quality assessment, and the results were reviewed and evaluated by the NTP Pathology Working Group (PWG). The final diagnoses represent a consensus of contractor pathologists and the PWG. Details of these review procedures have been described by Maronpot and Boorman (1982) and Boorman *et al.* (1985).

## Reproductive Toxicity

Sperm morphology and vaginal cytology examinations were performed for rats and mice administered formic acid at 0, 8, 32, and 128 ppm in the 13-week study. To screen for potential reproductive toxicity, epididymal sperm motility was evaluated at necropsy, and vaginal cytology was evaluated on animals during the 2 weeks just preceding necropsy, using procedures outlined by Morrissey *et al.* (1988). For the 12 days prior to sacrifice, females were subject to vaginal lavage with saline. The aspirated cells were air-dried onto slides, stained with Toluidine Blue O, and cover slipped. The relative preponderance of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were used to identify the stages of the estrual cycle.

Sperm motility was evaluated at necropsy as follows: The left epididymis was removed and quickly weighed; the cauda epididymis was removed at the junction of the vas deferens and the corpus epididymis, then weighed. Warm (37°C) Tyrodes buffer (mice) or test yolk buffer (rats) was applied to two pre-warmed slides, and a small cut made in the distal cauda epididymis. The sperm that extruded from the epididymis were dispersed throughout the solution, cover slipped, and counted immediately on a warmed microscope stage. Two independent observers counted the number of moving and non-moving sperm in 5 fields of 30 sperm or less per field. After sperm sampling for motility evaluation, the cauda was placed in phosphate buffered saline (PBS), gently chopped with a razor blade, and allowed to sit for 15 min. The remaining clumps of tissue were removed and the solution mixed gently and heat-fixed at 65°C. Sperm density was subsequently determined using a hemocytometer.

To quantify spermatogenesis, the left testis was weighed, frozen and stored. After thawing, testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the testis in PBS containing 10% DMSO. Homogenization-resistant spermatid nuclei were enumerated using a hemocytometer; the data were expressed as spermatid heads per total testis and per gram of testis.

## Genetic Toxicology

### Experimental Protocol

Testing was performed as described by Haworth *et al.* (1983). Formic acid was incubated with the *Salmonella typhimurium* tester strains TA97, TA98, TA100, and TA1535, either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley or Syrian hamster liver) for 20 minutes prior to the addition of soft agar supplemented with L-histidine and D-biotin, and subsequently plated on minimal glucose agar plates. Incubation continued for an additional 48 hours.

Each test consisted of triplicate plates of concurrent positive and negative controls and at least 5 doses of test chemical. High dose was limited by toxicity and did not exceed 3.33 mg/plate. All positive assays were repeated under the conditions which elicited the positive response.

A positive response was defined in this assay as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any single strain/activation combination. An equivocal response was defined as an increase in revertants which was neither dose-related, reproducible, or of sufficient magnitude to support a determination of mutagenicity. A negative response was obtained when no increase in revertant colonies was observed following chemical treatment.

## Statistical Methods

Two approaches were employed to assess the significance of pairwise comparisons between dosed and control groups in the analysis of continuous variables. Organ and body weight data, which are approximately normally distributed, were analyzed using the parametric multiple comparisons procedures of Williams (1971, 1972) and Dunnett (1955). Clinical chemistry and hematology data, which typically have skewed distributions, were analyzed using the nonparametric multiple comparisons methods of Shirley (1977) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of dose-response trends and to determine whether a trend-sensitive test (Williams, Shirley) was more appropriate for pairwise comparisons than a test capable of detecting departures from monotonic dose-response (Dunnett, Dunn). If the P-value from Jonckheere's test was greater than or equal to 0.10, Dunn's or Dunnett's test was used rather than Shirley's or Williams' test.

The outlier test of Dixon and Massey (1951) was employed to detect extreme values. No value selected by the outlier test was eliminated unless it was at least twice the next largest value or at most half of the next smallest value.

Because the vaginal cytology data are proportions (the proportion of the observation period that an animal was in a given estrous state), an arcsine transformation was used to bring the data into closer conformance with normality assumptions. Treatment effects were investigated by applying a multivariate analysis of variance (Morrison, 1976) to the transformed data to test for the simultaneous equality of measurements across dose levels.

## Quality Assurance

The 13-week toxicity studies of formic acid were performed in compliance with FDA Good Laboratory Practices regulations (21 CFR 58). The Quality Assurance Unit of Battelle Northwest Laboratories performed audits and inspections of protocols, procedures, data, and reports throughout the course of the studies. The operations of the Quality Assurance Unit were monitored by the NTP.

## TABLE 1 Experimental Design and Materials and Methods in the 14-Day and 13-Week Inhalation Studies of Formic Acid

| | |
|---|---|
| **Study Laboratory** | Battelle Pacific Northwest Laboratories |
| **Study Dates** | 14-Day Studies: August -- September, 1987<br>14-Day Dosed Feed Studies: December 1987 -- March, 1988 |
| **Strain and Species** | F344/N rats; B6C3$F_1$ mice |
| **Animal Source** | Taconic Farms, Inc., Germantown, NY |
| **Chemical Source** | BASF Wyandotte Corporation (Parsippany, NJ). |
| **Size of Study Groups** | 14-Day Studies:<br>5/sex/group of each species. Animals were individually caged.<br>13-Week Studies:<br>Mice--10/sex/group; rats--20/sex/group (10 core study and 10 for clinical pathology). Animals were individually caged. |
| **Method of Animal Distribution** | Animals assigned to dosed and control groups by computer-generated tables of random numbers, using body weight as a blocking variable. |
| **Route of Administration** | Whole body inhalation |
| **Exposure Concentrations** | 14-Day Studies: 0, 31, 62.5, 125, 250, and 500 ppm<br>13-Week Studies: 0, 8, 32, 64, and 128 ppm |
| **Diet** | NIH-07 available *ad libitum* except during exposure periods |
| **Animal Room Environment** | Temp--75 ± 3°F; relative humidity--55 ± 15%; fluorescent light 12 h/d; 15 ± 3 air changes/h. |
| **Time Held Before Study** | 14-Day Studies: Rats-11 d; Mice-12 d<br>13-Week Studies: Rats-12 d, Mice-13 d |
| **Age When Placed on Study** | 14-Day and 13-Week Studies: 6 wks (7 wks for mice in 13-week studies). |
| **Age When Killed** | 14-Day Studies: 8 wks<br>13-Week Studies: Rats-19 wks; Mice-20 wks |
| **Type and Frequency of Observation** | 14-Day Studies:<br>Observed 2 x d for mortality/moribundity; 2 x d each exposure day for clinical signs of toxicity; weighed on days 1, 8, and at necropsy.<br>13-Week Dosed Feed Studies:<br>Observed 2 x d for mortality/moribundity; body weights and clinical observations measured weekly and at necropsy. |

**Necropsy and Histologic Examinations (14-day studies)**

Necropsy was performed on all animals. The following tissues were examined microscopically: lungs, trachea, larynx, bronchial lymph nodes, nose (three transverse sections), and all gross lesions from all treated and control animals. Urinalysis, coagulation, serum chemistry were performed at day 3 and at termination.

**Necropsy and Histologic Examinations (13-week studies)**

Necropsy was performed on all animals. The following tissues were examined microscopically from all control and high dose groups: adrenal glands, brain, bronchial lymph nodes, cecum, colon, duodenum, epididymis/seminal vesicles/ prostate/testes or ovaries/uterus, esophagus, eyes (if grossly abnormal), femur (including marrow), gallbladder (mice), gross lesions and tissue masses with regional lymph nodes, heart, ileum, jejunum, kidneys, larynx, liver, lungs with mainstem bronchi, mammary gland and adjacent skin, mandibular and mesenteric lymph nodes, mediastinal lymph nodes, nasal cavity and turbinates, pancreas, parathyroid glands, pharynx (if grossly abnormal), pituitary gland, preputial /clitoral glands (rats), rectum, salivary glands, spinal cord and sciatic nerve (if neurologic signs present), spleen, stomach (including forestomach and glandular stomach), thigh muscle, thymus, thyroid gland, trachea, and urinary bladder. In addition to all gross lesions, the following tissues were examined in all other dose groups: rats--nose (three transverse sections), lung, larynx, trachea, bronchial and mediastinal lymph nodes; mice--nose (three transverse sections). Organ weights (to the nearest mg) were obtained from all core study animals and include: liver, thymus, right kidney, right testis, heart and lungs. Hematologic and serum chemical analyses were performed; sperm morphology and vaginal cytology were evaluated in rats and mice exposed to 0, 8, 32, and 128 ppm.

# RESULTS

## 2-Week Studies in Rats

One female and 3 male rats in the 500 ppm exposure groups died on day 10 of exposure (Table 2). Final body weights were significantly lower in male rats exposed to 250 and 500 ppm formic acid and in female rats exposed to 500 ppm, compared to control animals (Table 2). Exposure-related clinical signs were limited to the 250 and 500 ppm dose groups and were consistent with effects typically seen with respiratory irritants. Clinical signs noted included nasal discharge, increased preening, hypoactivity, and labored breathing. Male and female rats among the highest dose group developed corneal opacity.

**TABLE 2 Survival and Weight Gain of F344/N Rats in the 2-Week Inhalation Studies of Formic Acid**

| Exposure Concentration (ppm) | Survival[a] | Mean Body Weight (grams) Initial | Final | Change[b] | Final Weight Relative to Controls (%)[c] |
|---|---|---|---|---|---|
| **MALE** | | | | | |
| 0.0 | 5/5 | 119 | 177 | 58 | |
| 31.0 | 5/5 | 119 | 179 | 60 | 101 |
| 62.5 | 5/5 | 117 | 175 | 58 | 99 |
| 125.0 | 5/5 | 119 | 172 | 53 | 97 |
| 250.0 | 5/5 | 119 | 162 | 43 | 92 |
| 500.0 | 2/5 | 120 | 135 | 15 | 76 |
| **FEMALE** | | | | | |
| 0.0 | 5/5 | 94 | 127 | 33 | |
| 31.0 | 5/5 | 93 | 126 | 33 | 99 |
| 62.5 | 5/5 | 92 | 131 | 39 | 103 |
| 125.0 | 5/5 | 91 | 123 | 32 | 97 |
| 250.0 | 5/5 | 94 | 118 | 24 | 93 |
| 500.0 | 4/5 | 94 | 96 | 2 | 76 |

a Number surviving at 14 days/number of animals per dose group.
b Mean weight change of the animals in each dose group.
c (Dosed group mean/control group mean) x 100.

Effects of treatment on blood pH and concentrations of serum electrolytes were unremarkable. A mild, statistically significant increase occurred in concentrations of sodium in female rats in the highest exposure group (500 ppm). There did not appear to be any consistent effect of formic acid exposure on coagulation tests, as results of assays of prothrombin time and activated partial thromboplastin time did not differ among the groups (not shown). Results of urinalyses (performed after exposure day 3) indicated a reduction in 16-hour urine volumes in males and females exposed to 250 ppm and in males exposed to 500 ppm; urine specific gravity was variably increased in exposed males and females, and correlated with reductions in urine volume. Similarly, concentrations of glucose and protein, and activities of AST, GGT and AP were increased, but when corrected for total 16-hour excretion, they were unchanged from controls.

At necropsy, exposure-related gross lesions consisted of dried exudate around the external (anterior) nares in 3 males and 3 females from the 500 ppm exposure groups. Although cloudiness of the cornea was observed clinically at this exposure concentration during the course of the study, corneal opacity was identified in only 1 male rat at the time of necropsy.

This corneal change was characterized microscopically by a very minimal inflammatory cell infiltrate (neutrophils).

Both absolute and relative thymus weights were significantly less (as much as 50% in male and female rats exposed to 500 ppm) compared to controls. There were no differences in other absolute organ weights between exposed and control animals. The relative weights of the kidney in males and females, and of the heart in females, were increased significantly in high-dose animals compared to controls; however, the group mean body weights of these animals were lower than controls, which contributed to these differences.

Histopathologic lesions related to formic acid exposure in the upper respiratory tract were similar in nature, and dose-related in incidence and severity, in male and female rats exposed at concentrations of 62.5 ppm or higher (Table 3). With exposure concentrations of 125 ppm or higher, lesions occurred in the respiratory and olfactory epithelium in the anterior (Level I) and mid portion (Level II) of the nasal cavity. Lesions were most severe at the 500 ppm exposure level; squamous metaplasia and necrosis of the respiratory and olfactory epithelium were present in all rats. An inflammatory cell (neutrophils) infiltrate was present in the mucosa, and exudate was present in the nasal cavity (Plates 1, 2). At this highest exposure concentration, squamous metaplasia of the larynx occurred in 1 male and 1 female rat. Microscopic lesions in rats exposed to 250 ppm were slightly less severe than in the 500 ppm group; inflammation and squamous metaplasia of the larynx were not present at this exposure concentration.

**TABLE 3 Histopathologic Lesions in F344/N Rats in the 2-Week Inhalation Studies of Formic Acid**

| | Exposure Concentration (ppm) | | | | | |
|---|---|---|---|---|---|---|
| Site/Lesion | 0 | 31 | 62.5 | 125 | 250 | 500 |
| **MALE** | | | | | | |
| **Nose** | | | | | | |
| **Respiratory epithelium** | | | | | | |
| squamous metaplasia | 0 | 0 | 4 (1.3)[a] | 5 (1.8) | 5 (2.8) | 5 (2.6) |
| inflammation | 0 | 0 | 0 | 3 (1.0) | 5 (2.4) | 5 (3.0) |
| necrosis | 0 | 0 | 0 | 0 | 5 (2.0) | 5 (2.6) |
| **Olfactory epithelium** | | | | | | |
| necrosis | 0 | 0 | 0 | 1 (1.0) | 2 (2.5) | 5 (2.6) |
| **Larynx** | | | | | | |
| squamous metaplasia | 0 | 0 | 0 | 0 | 0 | 1 (1.0) |
| inflammation | 0 | 0 | 0 | 0 | 0 | 2 (1.5) |
| **FEMALE** | | | | | | |
| **Nose** | | | | | | |
| **Respiratory epithelium** | | | | | | |
| squamous metaplasia | 0 | 0 | 3 (1.6) | 5 (2.6) | 5 (3.0) | 5 (3.0) |
| inflammation | 0 | 0 | 0 | 4 (1.3) | 5 (2.0) | 5 (3.0) |
| necrosis | 0 | 0 | 0 | 0 | 3 (1.6) | 5 (3.0) |
| **Olfactory epithelium** | | | | | | |
| necrosis | 0 | 0 | 0 | 1 (1.0) | 4 (1.5) | 5 (3.0) |
| **Larynx** | | | | | | |
| squamous metaplasia | 0 | 0 | 0 | 0 | 0 | 1 (1.0) |
| inflammation | 0 | 0 | 0 | 0 | 1 (1.0) | 1 (2.0) |

[a] Incidence and severity score ( ) based on a scale of 1 to 4: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked. Scores are averages based on the number of animal with lesions from groups of 5.

Rats exposed to 125 ppm formic acid had a decreased severity and incidence of nasal lesions when compared to those in the higher exposure groups; histopathologic lesions generally consisted of a minimal-to-mild squamous metaplasia of the respiratory epithelium on the nasal septum, lateral walls, and tips of the nasoturbinates (Plate 3). Minimal focal necrosis of the olfactory epithelium occurred in 2 rats at this exposure concentration. In the 62.5 ppm exposure groups, lesions were limited to the most anterior nasal section (Level I) and consisted of minimal-to-mild squamous metaplasia of the respiratory epithelium (Table 3). There were no microscopic lesions in the olfactory epithelium at this exposure concentration, and no treatment-related lesions in rats exposed to 31.5 ppm formic acid. No lesions in the lower respiratory tract were considered related to formic acid exposure at any concentration studied.

## 13-Week Studies in Rats

All rats survived to the end of the studies. Male rats exposed to 32 ppm formic acid had a mild but significant increase in final body weight compared to control animals (Table 4 and Figure 1). Similarly, body weight gains were significantly greater in male rats exposed to 16, 32, and 64 ppm formic acid compared to control animals (Table 4 and Figure 1). No clinical signs that were clearly exposure-related were noted during the studies.

**TABLE 4 Survival and Weight Gain of F344/N Rats in the 13-Week Inhalation Studies of Formic Acid**

| Exposure Concentration (ppm) | Survival[a] | Mean Body Weight (grams) | | | Final Weight Relative to Controls (%)[c] |
|---|---|---|---|---|---|
| | | Initial | Final | Change[b] | |
| **MALE** | | | | | |
| 0 | 10/10 | 119 | 339 | 220 | |
| 8 | 10/10 | 124 | 357 | 233 | 105 |
| 16 | 10/10 | 117 | 357 | 240 | 105 |
| 32 | 10/10 | 123 | 367 | 244 | 108 |
| 64 | 10/10 | 119 | 362 | 243 | 107 |
| 128 | 10/10 | 121 | 333 | 212 | 98 |
| **FEMALE** | | | | | |
| 0 | 10/10 | 113 | 212 | 99 | |
| 8 | 10/10 | 113 | 210 | 97 | 99 |
| 16 | 10/10 | 112 | 205 | 93 | 97 |
| 32 | 10/10 | 111 | 208 | 97 | 98 |
| 64 | 10/10 | 111 | 205 | 94 | 97 |
| 128 | 10/10 | 108 | 201 | 93 | 95 |

a Number surviving at 13 weeks/number of animals per dose group.
b Mean weight change of the animals in each dose group.
c (Dosed group mean/Control group mean) x 100.

Changes in hematologic variables were few and generally minimal to mild in magnitude. Increases in white blood cell (WBC) counts in male and female rats at 3 days were produced by mild lymphocytoses. RBC counts were significantly increased in male rats in the 2 highest exposure groups at day 3. Although there were no statistically significant changes in WBC counts at the 13-week time point, neutrophil counts were decreased in male and female rats in all exposure groups. The decreases were mild to moderate and not dependent on the exposure concentration. In the female rats at 23 days, mild but significant increases in MCH and MCV were produced by minimal

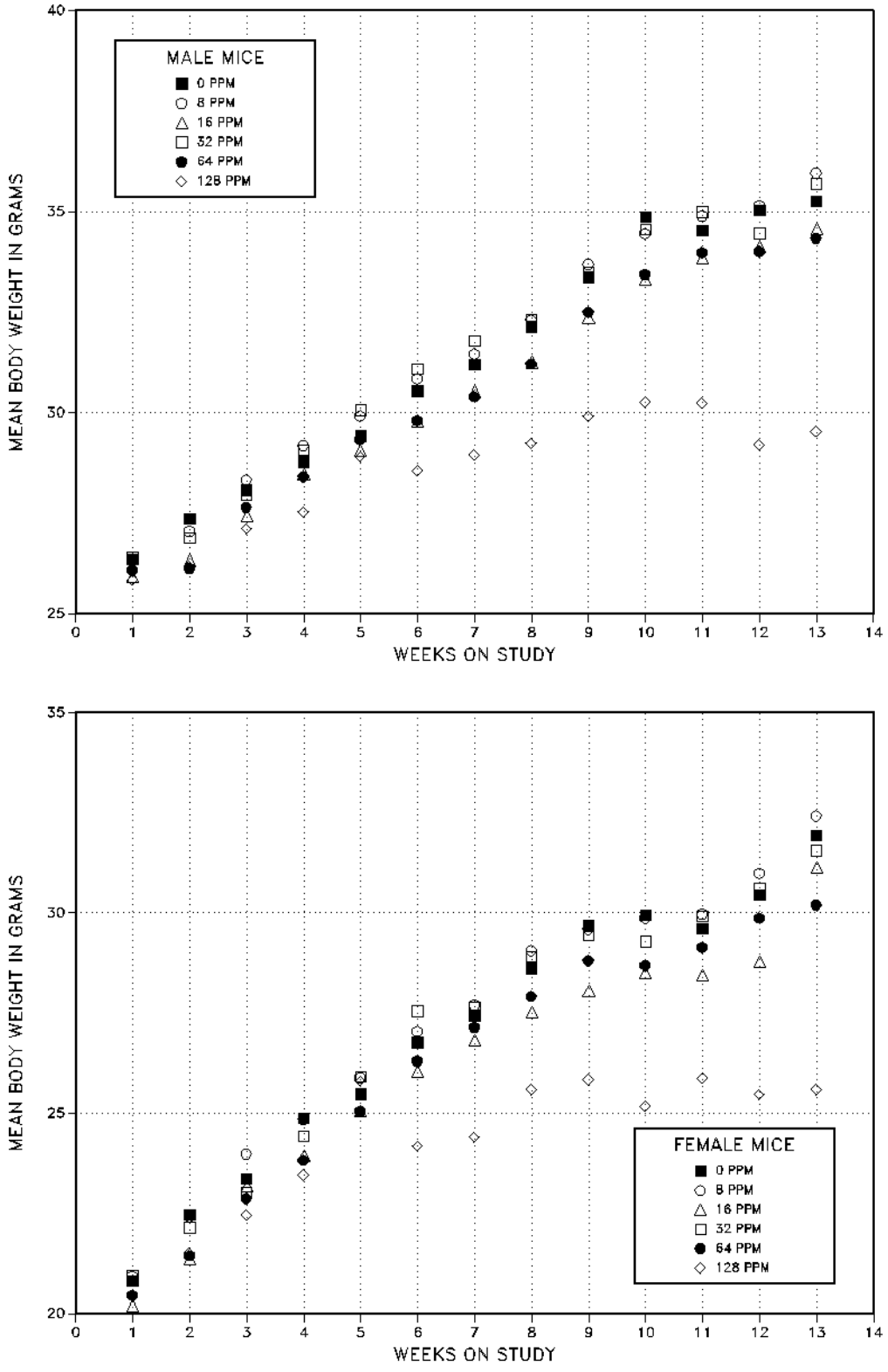


**Figure 1 Body Weights of F344/N Rats in the 13-Week Inhalation Studies of Formic Acid**

to mild decreases in RBC counts. In female rats at 13 weeks, there were minimal but significant increases in MCHC in animals at all exposure concentrations, produced by increases in HGB concentrations that were occasionally significant. Minimal but significant decreases in MCV in female rats in 2 exposure groups (16 and 128 ppm) at 13 weeks were associated with increases of similar magnitude in RBC counts.

There were mild, significant decreases in concentrations of serum albumin in female rats at day 3 (32, 64, and 128 ppm exposure groups) and increases in male rats at 13 weeks (8, 16, and 32 ppm exposure groups). Concentrations of total serum protein were decreased in female rats in all exposure groups at day 3. Male and female rats exposed to 16, 32 (female only), 64, and 128 ppm formic acid had significant increases in serum AP at 13 weeks. Additional changes in serum biochemical variables in rats exposed to formic acid included decreases in activities of amylase (female rats, days 3 and 23) and CK (male rats, day 3; female rats, day 23), increases in activities of SDH (male rats, day 3), and decreases in concentrations of UN and creatinine (male and female rats, day 3).

There were no unusual gross lesions noted at necropsy. Liver weights were somewhat greater in male rats in all exposure groups and liver-to-body-weight ratios (relative weights) were increased in male rats exposed to 32, 64, and 128 ppm formic acid (Appendix A). Absolute and relative lung weights were decreased in all exposed groups of female rats. In male rats, relative lung weights were decreased in all exposure groups, and absolute weights were decreased in the 64 and 128 ppm groups.

Microscopic changes attributed to formic acid exposure occurred in the respiratory and olfactory epithelium of the nose and generally were limited to the 128 ppm exposure groups (Table 5).

**TABLE 5 Histopathologic Lesions in F344/N Rats in the 13-Week Inhalation Studies of Formic Acid**

| | Exposure Concentration (ppm) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 8 | 16 | 32 | 64 | 128 |
| **Site/Lesion** | | | | | | |
| **MALE** | | | | | | |
| **NOSE** | | | | | | |
| **Respiratory epithelium** | | | | | | |
| squamous metaplasia | 0 | 0 | 0 | 0 | 0 | 9 (1.0)[a] |
| **Olfactory epithelium** | | | | | | |
| degeneration | 0 | 0 | 0 | 1 (1.0) | 1 (1.0) | 9 (1.2) |
| **FEMALE** | | | | | | |
| **NOSE** | | | | | | |
| **Respiratory epithelium** | | | | | | |
| squamous metaplasia | 0 | 0 | 0 | 0 | 0 | 6 (1.4) |
| **Olfactory epithelium** | | | | | | |
| degeneration | 0 | 0 | 0 | 0 | 0 | 5 (1.0) |

a Incidence and severity score ( ) based on a scale of 1 to 4: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked. Scores are averages based on the number of animals with lesions from groups of 10.

Changes in the respiratory epithelium consisted of a minimal squamous metaplasia in which the pseudostratified, ciliated columnar cells were replaced by a flattened, non-ciliated epithelium varying from approximately 2 to 5 cells in thickness. A few inflammatory cells sometimes were associated with these areas of metaplasia, but inflammation was not a prominent feature of the nasal lesions. Squamous metaplasia occurred most often in the respiratory epithelium that lines the most dorsal portion of the dorsal meatus in the nose's anterior section (Level I). Foci of squamous metaplasia occasionally were present on the anterior nasal septum and/or tips (margins) of the nasoturbinates (Plate 4). In the olfactory epithelium, degenerative changes were minimal to mild and generally limited to the area of the dorsal meatus in the mid-nasal section (Level II). Degeneration was characterized by a loss of the usual orderly arrangement of the pseudostratified layer of nuclei and by a slight reduction in the normal thickness of the olfactory epithelium. This decreased thickness was the result of a reduction in the amount of the cytoplasm at the apical portion of the olfactory epithelial cells and a decrease in the number of sensory and sustentacular cell nuclei (Plates 5, 6). An increase in the basophilic staining of some nuclei was seen, and, in a few cells, the nucleus appeared pyknotic, or fragmented; however, necrosis was not a characteristic feature of the olfactory lesion. There was no evidence of metaplasia in the olfactory epithelium or atrophy of the nerve fibers in the olfactory mucosa.

In 19/20 male and female rats from the control and 32 ppm exposure groups there were minimal to mild inflammatory lesions in the lung consisting of aggregates of macrophages and/or neutrophils in alveoli and hyperplasia of peribronchiolar lymphoid tissues and alveolar epithelium. These pulmonary lesions, which were generally less severe in females, were limited to the control and mid-dose groups and corresponded to the slightly greater lung weights observed for these groups of rats.

There were no effects of exposure to formic acid on measures of sperm motility, density, or testicular or epididymal weights, and no changes were seen in the length of the estrous cycle (Appendix C).

# Plates

**Plate 1.** Nasoturbinate from male rat exposed to 500 ppm formic acid in the 2-week study shows prominent squamous metaplasia (M) of the respiratory mucosa, and inflammatory cell exudate (E) on the mucosal surface. 330X

**Plate 2.** Medial septum of male rat exposed to 500 ppm formic acid in the 2-week study shows squamous metaplasia (M) of the respiratory mucosa, with keratin and an inflammatory cell exudate (arrows) along the mucosal surface. 330X

**Plate 3.** Nasal turbinate of female rat exposed to 125 ppm formic acid in the 2-week study shows minimal squamous metaplasia of respiratory mucosa on tip of turbinate (arrows). Compare with Plate 4, showing similar nasal lesion at same dose after 13 weeks of exposure. 330 X

**Plate 4.** Nasal turbinate of female rat exposed to 128 ppm formic acid in the 13-week study shows minimal squamous metaplasia of the respiratory mucosa on the tip of turbinate (arrows). Note the similar severity to that seen in Plate 3 with the same dose in the 2-week study. 330X

**Plate 5.** Olfactory mucosa from dorsal meatus of female rat exposed to 128 ppm formic acid in the 13-week study shows degeneration of the olfactory epithelial layer. Note the thinning of the apical cytoplasm (arrows) and slight decreased thickness of the nuclear layer compared to the control in Plate 6. 330X

**Plate 6.** Olfactory mucosa from dorsal meatus of control female rat in the 13-week study, for comparison with minimal degeneration in Plate 5. 330X

**Plate 7.** Nasal turbinate from female mouse exposed to 125 ppm formic acid in the 2-week study shows minimal squamous metaplasia of respiratory epithelium on turbinate. Compare to normal turbinate from control in Plate 8. 330 X

**Plate 8.** Nasal turbinate from control female mouse in the 2-week study shows normal cuboidal to columnar, ciliated (arrows) respiratory epithelium. 330X

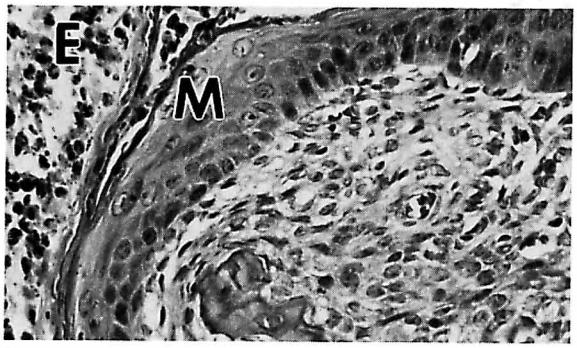


Plate 1

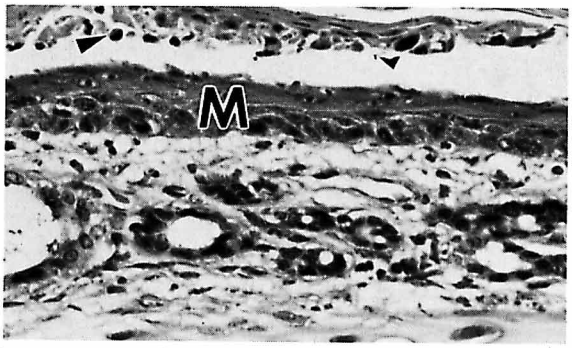


Plate 2

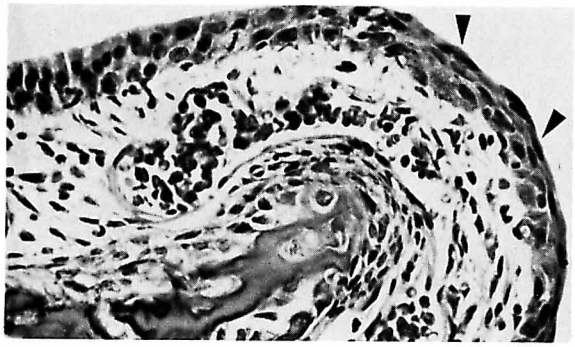

Plate 3

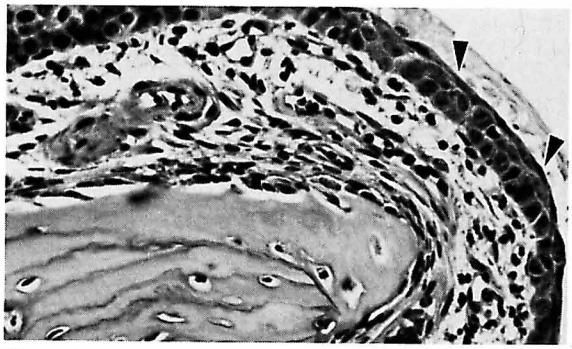

Plate 4

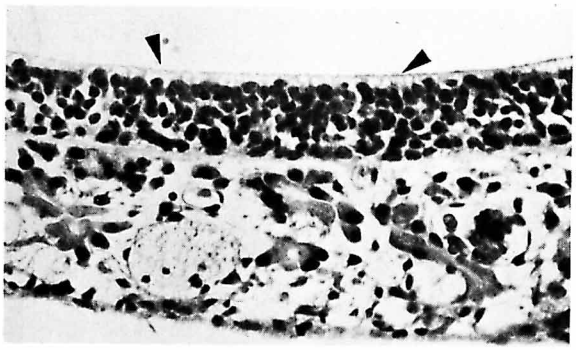

Plate 5

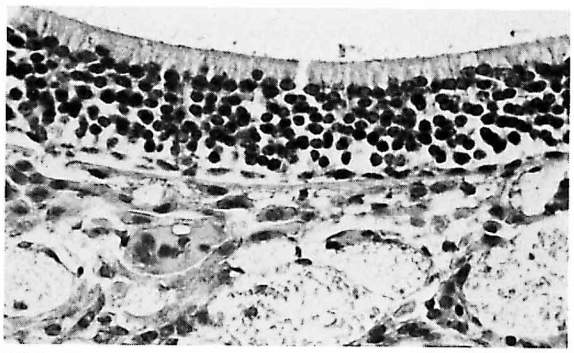

Plate 6

## 2-Week Studies in Mice

All mice exposed to 500 ppm of formic acid died during the first week of study; a female mouse from the 250 ppm exposure group became moribund and was killed on day 4. At the end of the study, body weight gain was significantly less than controls in the 250 ppm exposure groups (Table 6). Exposure-related clinical signs were limited to the 250 and 500 ppm exposure groups. These signs were consistent for effects produced by respiratory irritants and included nasal discharge, increased preening, and labored breathing. Corneal opacity was present in the highest dose groups of male and female mice.

**TABLE 6 Survival and Weight Gain of B6C3F$_1$ Mice In the 2-Week Inhalation Studies of Formic Acid**

| Exposure Concentration (ppm) | Survival[a] | Mean Body Weight (grams) | | | Final Weight Relative to Controls (%)[c] |
|---|---|---|---|---|---|
| | | Initial | Final | Change[b] | |
| **MALE** | | | | | |
| 0.0 | 5/5 | 22.8 | 25.8 | 3.0 | |
| 31.0 | 5/5 | 22.6 | 25.3 | 2.7 | 98 |
| 62.5 | 5/5 | 22.7 | 25.5 | 2.8 | 99 |
| 125.0 | 5/5 | 23.0 | 24.7 | 1.7 | 96 |
| 250.0 | 5/5 | 23.1 | 21.0 | -2.1 | 81 |
| 500.0 | 0/5 | 22.6 | - | - | - |
| **FEMALE** | | | | | |
| 0.0 | 5/5 | 19.6 | 22.5 | 2.9 | |
| 31.0 | 5/5 | 19.5 | 22.5 | 3.0 | 100 |
| 62.5 | 5/5 | 19.6 | 22.7 | 3.1 | 101 |
| 125.0 | 5/5 | 19.8 | 21.5 | 1.7 | 96 |
| 250.0 | 4/5 | 19.2 | 18.9 | -0.3 | 84 |
| 500.0 | 0/5 | 19.8 | - | - | - |

a Number surviving at 14 days/number of animals per dose group.
b Mean weight change of the animals in each dose group.
c (Dosed group mean/control group mean) x 100.

At necropsy, exposure-related gross lesions consisted of dried exudate around the external nares of all mice from the 500 ppm exposure groups and the 1 female from the 250 ppm exposure groups that died during the first week of the study. In mice that died during the study, segmental portions of the gastrointestinal tract (stomach and small intestine) were distended with air. Distention is attributed to the swelling and occlusion of nasal passages and subsequent swallowing of air which occurs when an obligate nose-breathing animal must breathe by mouth. There were no exposure-related gross lesions in mice necropsied at the end of the study. There were small increases (~10%) in the relative kidney weight in males exposed to 62.5, 125, and 250 ppm and in females exposed to 250 ppm. Thymus weights were reduced on an absolute and relative basis in mice exposed to 250 ppm; relative lung weights were increased mildly in these groups.

Histopathologic lesions were similar in male and female mice. They were limited to the nasal passages, except at the highest dose where they also were present in the larynx, pharynx, and trachea (Table 7). At 500 ppm, exposure-related lesions were of greatest severity in the most anterior section (Level I) of the nose and consisted of necrosis of the respiratory epithelium, with an accumulation of inflammatory cells in the mucosa and lumen of the nasal cavity. Squamous metaplasia of the respiratory epithelium generally was not present, but occasionally a basophilic-

**TABLE 7 Histopathologic Lesions in B6C3F$_1$ Mice in the 2-Week Inhalation Studies of Formic Acid**

| | Exposure Concentration (ppm) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 31 | 62.5 | 125 | 250 | 500 |
| **MALE** | | | | | | |
| **Nose** | | | | | | |
| **Respiratory epithelium** | | | | | | |
| squamous metaplasia | 0 | 0 | 0 | 3 (1.3)[a] | 4 (1.3) | 1 (1.0) |
| inflammation | 0 | 0 | 0 | 2 (1.0) | 4 (1.2) | 5 (1.4) |
| necrosis | 0 | 0 | 0 | 0 | 0 | 4 (3.5) |
| **Olfactory epithelium** | | | | | | |
| degeneration | 0 | 0 | 0 | 0 | 3 (1.3) | 1 (2.0) |
| necrosis | 0 | 0 | 0 | 0 | 0 | 3 (2.0) |
| **Larynx** | | | | | | |
| squamous metaplasia | 0 | 0 | 0 | 0 | 0 | 5 (2.8) |
| inflammation | 0 | 0 | 0 | 0 | 0 | 3 (1.0) |
| **Pharynx** | | | | | | |
| necrosis | 0 | 0 | 0 | 0 | 0 | 3 (2.0) |
| **FEMALE** | | | | | | |
| **Nose** | | | | | | |
| **Respiratory epithelium** | | | | | | |
| squamous metaplasia | 0 | 0 | 2 (1.0) | 3 (1.3) | 4 (1.0) | 0 |
| inflammation | 0 | 0 | 0 | 2 (1.5) | 5 (1.4) | 5 (1.8) |
| necrosis | 0 | 0 | 0 | 0 | 2 (1.5) | 5 (3.6) |
| **Olfactory epithelium** | | | | | | |
| degeneration | 0 | 0 | 0 | 0 | 2 (2.0) | 0 |
| necrosis | 0 | 0 | 0 | 0 | 1 (1.0) | 5 (1.8) |
| **Larynx** | | | | | | |
| squamous metaplasia | 0 | 0 | 0 | 0 | 0 | 1 (2.0) |
| inflammation | 0 | 0 | 0 | 0 | 0 | 3 (1.0) |
| necrosis | 0 | 0 | 0 | 0 | 0 | 5 (2.2) |
| **Pharynx** | | | | | | |
| necrosis | 0 | 0 | 0 | 0 | 0 | 2 (1.0) |

[a] Incidence and severity score ( ) based on a scale of 1 to 4: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked. Scores are averages based on the number of animals with lesions from groups of 5.

staining, flattened cuboidal cell partially covered the mucosal surface in areas with necrosis. Morphologic features of these cells were suggestive of regeneration; this was more prominent in the mice which survived longer.

Necrosis of respiratory and olfactory epithelium and nasal turbinate bone also was present in the midsection (Level II) of the nose. Focal areas of necrosis with associated inflammation and ulceration were present on the anterior portion of the pharyngeal hard palate. In female mice, there was necrosis, inflammation, and squamous metaplasia of the respiratory epithelium in the larynx. In the larynx of male mice, necrosis was not present, but squamous metaplasia was more prominent than in females. In the trachea of 2 of the 5 males from this exposure group, there was focal regeneration of the respiratory epithelium, morphologically similar to that seen in the nasal cavity. Mice exposed to 250 ppm of formic acid had a lower incidence and severity of necrosis of respiratory epithelium and nasal turbinate bone; in mice from this exposure concentration, necrosis of the olfactory epithelium was present in only a single female. There was a minimal to mild degeneration of the olfactory epithelium similar to that previously described for the rats. This degeneration was characterized by a decrease in thickness and loss of the normal arrangement of the pseudostratified nuclear layers comprising the olfactory

epithelium. Squamous metaplasia of the respiratory epithelium was more prominent in the 250 ppm exposure groups when compared to the 500 ppm groups which died during the first week of exposure. At an exposure concentration of 125 ppm of formic acid, squamous metaplasia and inflammation were present in 3 males and 3 females (Plates 7, 8); necrosis (minimal) of the respiratory epithelium was present in 1 female. There was no microscopic evidence for toxicity in the olfactory epithelium at this exposure concentration. There were no exposure-related changes in male mice exposed to concentrations below 125 ppm. At 62.5 ppm, there was minimal squamous metaplasia of the respiratory epithelium in 2 female mice; no microscopic evidence of toxicity was present in the 31 ppm exposure group of female mice.

## 13-Week Studies in Mice

There were no clinical signs or mortality associated with exposure of male or female mice to formic acid concentrations up to 128 ppm. Body weight gains were significantly less than controls in the 128 ppm exposure groups of both sexes and in female mice exposed to 64 ppm formic acid (Table 8, Figure 2). Changes in organ weights were limited largely to increases in relative weights in animals in the 128 ppm groups (Appendix A). This was primarily a reflection of the lower body weights of these animals compared to controls, and of the greater relative weight of organs in smaller animals. However, small increases in relative liver and kidney weights were seen in males and females, respectively, in the 32 and 64 ppm exposure groups.

**TABLE 8 Survival and Weight Gain of B6C3F$_1$ Mice in the 13-Week Inhalation Studies of Formic Acid**

| Exposure Concentration (ppm) | Survival[a] | Mean Body Weight (grams) | | | Final Weight Relative to Controls (%)[c] |
|---|---|---|---|---|---|
| | | Initial | Final | Change[b] | |
| **MALE** | | | | | |
| 0 | 10/10 | 26.4 | 35.3 | 8.9 | |
| 8 | 10/10 | 26.3 | 36.0 | 9.7 | 102 |
| 16 | 10/10 | 25.9 | 34.6 | 8.7 | 98 |
| 32 | 10/10 | 26.4 | 35.7 | 9.3 | 101 |
| 64 | 10/10 | 26.1 | 34.3 | 8.2 | 97 |
| 128 | 9/10 | 25.9 | 29.5 | 3.6 | 84 |
| **FEMALE** | | | | | |
| 0 | 10/10 | 20.8 | 31.9 | 11.1 | |
| 8 | 10/10 | 21.0 | 32.4 | 11.4 | 102 |
| 16 | 10/10 | 20.2 | 31.1 | 10.9 | 97 |
| 32 | 10/10 | 21.0 | 31.6 | 10.6 | 99 |
| 64 | 10/10 | 20.5 | 29.9 | 9.4 | 94 |
| 128 | 9/10 | 20.9 | 25.6 | 4.7 | 80 |

a Number surviving at 13 weeks/number of animals per dose group.
b Mean weight change of the animals in each dose group.
c (Dosed group mean/Control group mean) x 100.

There were no exposure-related gross lesions; microscopic changes attributed to toxicity of formic acid were limited to degeneration of the olfactory epithelium of the nose in a few mice from the 64 and 128 ppm exposure groups (Table 9). This minimal degeneration occurred in the dorsal portion of the dorsal meatus in the anterior or mid-nasal section (Levels I and II) and was similar to the olfactory degeneration described previously.

**TABLE 9 Histopathologic Lesions in B6C3F$_1$ Mice in the 13-Week Inhalation Studies of Formic Acid**

| | Exposure Concentration (ppm) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 8 | 16 | 32 | 64 | 128 |
| **MALE** | | | | | | |
| **Olfactory epithelium** degeneration | 0 | 0 | 0 | 0 | 0 | 2 (1.0)[a] |
| **FEMALE** | | | | | | |
| **Olfactory epithelium** degeneration | 0 | 0 | 0 | 0 | 2 (1.0) | 5 (1.0) |

[a] Incidence and severity score ( ) based on a scale of 1 to 4: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked. Scores are averages based on the number of animals with lesions from groups of 10.

There were no adverse effects of formic acid exposure on reproductive parameters evaluated in male or female mice (Appendix C). Sperm motility was somewhat lower in the exposed groups compared to controls, but the values for controls were rather high, and the values for exposed mice fall well within the historical range for control mice.

## Genetic Toxicity

Buffered solutions of formic acid were found not mutagenic in *Salmonella typhimurium* strains TA100, TA1535, TA97, and TA98, (Appendix D1).

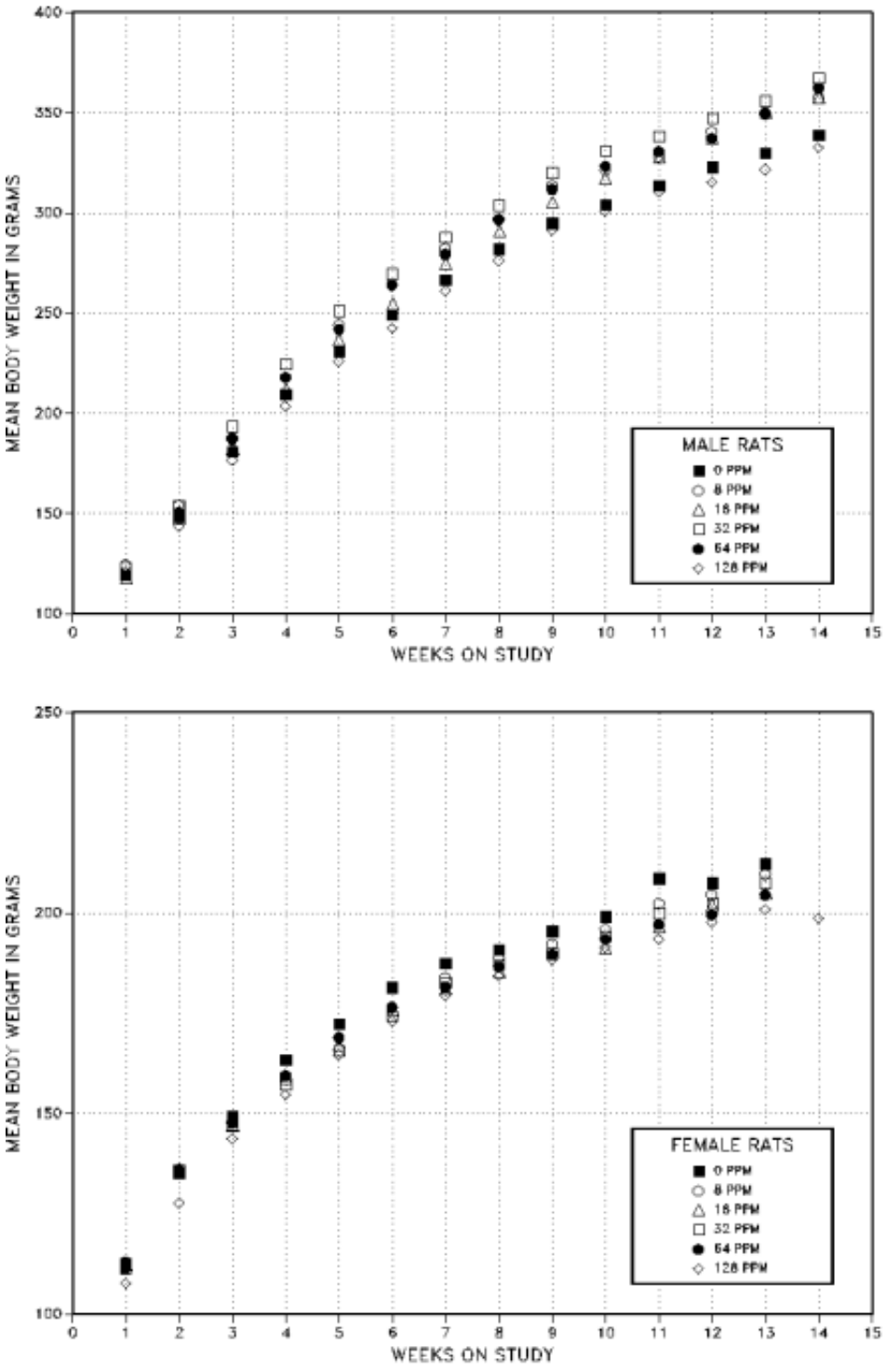


**Figure 2 Body Weights of B6C3F$_1$ Mice in the 13-Week Inhalation Studies of Formic Acid**

# DISCUSSION

The upper respiratory tract was the site for toxicity in rats and mice following exposure to formic acid by the inhalation route. In 2-week studies, toxicity, as evidenced primarily by necrosis and inflammation, was limited to the nasal passages, pharynx, larynx, and trachea of rats and mice exposed to concentrations of 250 and 500 ppm formic acid. Mice generally were more sensitive to the toxicity of formic acid at the highest exposure concentrations. Deaths were attributed to swelling of the nasal mucosa resulting in marked impairment of respiration. Because of mortality, clinical signs of labored breathing, and depression in body weight gain seen at exposure concentrations of 250 and 500 ppm in the 2-week studies, the highest exposure for the 13-week studies was limited to 128 ppm.

Microscopic lesions following exposure to 125 ppm formic acid for 2 weeks were limited to the nasal respiratory and olfactory epithelium in rats and the respiratory epithelium in mice. At the end of the 13-week studies there was little evidence for progression (in severity or incidence) of the respiratory or olfactory lesions at exposure concentrations equivalent to those in the 2-week studies. Exposure-related toxicity in the larynx, pharynx, or trachea that was seen in rats and mice at the 250 and 500 ppm exposure concentrations in the 2-week studies did not occur following 13 weeks of exposure to concentrations as high as 128 ppm.

Rats exposed to equivalent concentrations of formic acid for 2 weeks and 13 weeks had no increased severity of lesions in the nasal passages following the longer period of exposure. In fact, the minimal effects present after 2 weeks of exposure to 62.5 ppm formic acid were not evident in male or female rats exposed to 64 ppm for 13 weeks; and necrosis of the respiratory epithelium observed in rats after 2 weeks exposure to 125 ppm was not present in the 13-week study among rats in the 128 ppm exposure group. Squamous metaplasia of the respiratory epithelium was present after 13 weeks of exposure to 128 ppm formic acid, but the severity was equal to or less than that present at 2 weeks. After 13 weeks there was no evidence of hyperplasia, dysplasia, or development of a superficial layer of keratinized epithelium in the areas of squamous metaplasia. Adaptation of nasal respiratory epithelium to the irritant effects of formaldehyde vapor has been observed in the rat (Monticello, 1990). Six weeks of exposure to a vapor concentration of 6 ppm resulted in squamous metaplasia and hyperplasia of the respiratory epithelium. When the period of exposure was increased to 18 months, treatment-related microscopic lesions were no longer present.

Although olfactory epithelial necrosis was not present in the 13-week study, minimal degeneration of olfactory epithelium was present in most rats in the highest exposure groups. Absence of inflammation, hyperplasia, metaplasia, and nerve fiber atrophy in the olfactory mucosa is further indication of the minimal severity of the olfactory degeneration. There were no lesions in the lungs attributed to formic acid toxicity; the slightly greater absolute and relative lung weights which occurred in the control and the 32-ppm exposure groups were associated with minimal to mild inflammatory lesions of undetermined etiology. Morphologically identical inflammatory lesions with corresponding increased lung weights have been seen in control and treated rats from other toxicity studies (NTP, 1992); the occurrence of these lesions has not been related to chemical exposure in either incidence or severity.

In mice the squamous metaplasia of respiratory epithelium which developed after 14 days of exposure to formic acid at 125 ppm was not present after 13 weeks of exposure to 128 ppm. In contrast, however, the minimal olfactory degeneration in 7/20 mice exposed to 128 ppm for 13 weeks did not develop in mice exposed to a similar concentration (125 ppm) of formic acid during the shorter period of the 2-week study.

Hematologic changes in rats measured at 3 time points during the study were mild and generally unremarkable. Changes related to RBC variables could have been associated with minimal to mild hemoconcentration. Considering the changes in WBC variables, the lymphocytosis at day 3 of exposure is consistent with a physiologic response, as opposed to an immunologic response. Leukocytoses produced by endogenous release of epinephrine can result in increases in lymphocyte counts. However, these usually are accompanied by neutrophilia. An explanation for the neutropenia at 13 weeks (not dose-related) is not obvious. Causes of neutropenias include decreased production in the bone marrow, increased margination or sequestration in the peripheral circulation, and increased utilization by an inflammatory response. Based on histopathologic findings at 13 weeks, an inflammatory response does not appear to be an adequate explanation.

Changes in serum biochemical variables were few and mild. Decreases in concentrations of UN, albumin (and consequently, total protein), and creatinine at day 3 are consistent with a decreased intake of food. An increased concentration of albumin, as occurred in male rats at 13 weeks, is usually associated with hemoconcentration (mild). At 3 days, the increase in SDH activity in male rats exposed to formic acid indicates damage to hepatocytes. However, the lack of increase in other indicators of hepatocellular injury (ALT, bile acids) and the extent of the increase in activity of SDH suggest a minimal to mild effect. Increases in serum activities of ALP (13 weeks, male and female rats) generally are produced by disorders in bone, liver, or intestines. However, microscopic changes were not observed in these tissues, and the biologic importance of this finding, as well as of the decreases in activities of amylase (females) and CK (males), are not known.

The site-specific and morphological effects of formic acid on the upper respiratory tract in rats and mice are consistent with those produced by exposure to irritant chemicals administered by the inhalation route (Buckley *et al.,* 1984; Boorman *et al.,* 1987; Morgan *et al.,* 1990; Jiang *et al.,* 1983). A spectrum of histopathologic lesions in the upper respiratory tract after exposure to water-soluble irritant chemicals has been described (Morgan *et al.,* 1990; Buckley *et al.,* 1984). As seen in this study, the squamous epithelium lining the anterior portion of the nasal cavity typically is more resistant to the toxic effect of irritant gases, but lesions frequently occur at multiple locations in the anterior nasal cavity including the dorsal meatus, nasal septum, and the tips or margins of the nasoturbinates (Morgan *et al.,* 1990). Irritant chemicals administered at sufficiently high concentrations result not only in toxicity in the nasal cavity but at sites lower in the respiratory tract, including the pharynx, larynx, trachea, and lung (Boorman *et al., 1*987; Jiang *et al.,* 1983).

Aerosols containing particulates (dusts), including cobalt sulfate (Bucher *et al.,* 1990) and nickel compounds (Dunnick *et al.,* 1989), also have produced a similar spectrum of toxicity in the nasal

cavity. Strong chemical irritants may produce marked necrosis, inflammation, or metaplasia at these sites as well as in the nasal cavity; inflammatory exudate or swelling of respiratory tract tissues may result in dyspnea or death. Necrosis of the turbinate bone seen in the 2-week studies in mice is consistent with changes described for other chemicals that have caused extensive necrosis and ulceration of the respiratory mucosa on the nasal turbinates (Gross *et al.,* 1987; Monticello *et al.,* 1990). Similarly, irritant gases result in degenerative changes of the olfactory epithelium, most commonly in the most anterior portion of the dorsal meatus (Gaskell, 1990), the same site in the nose where olfactory degeneration occurred with exposure to formic acid. Despite the clinical signs (marked toxicity and mortality at the highest exposure concentrations of formic acid in the 2-week studies), at 125 ppm and below there were minimal histopathologic effects related to exposure. When compared with findings in the 2-week study, the incidence and severity of histopathologic changes in the respiratory epithelium of the nasal cavity from the 125 ppm exposure concentration groups suggest some adaptation to the irritant effects, following resolution of the initial injury. However, there appears to be less evidence of adaptation in the olfactory mucosa where, after 13 weeks, minimal degeneration occurred at a dose level (125 ppm) where no effect was seen in mice and only a minimal effect was seen in 2/10 rats following 2 weeks of exposure.

The lack of significant systemic effects from exposure of rats and mice to formic acid should be considered in light of the known differences in species susceptibilities to methanol toxicity. After ingestion or administration of methanol, human beings and primates can develop severe metabolic acidosis and blindness. Rodents are resistant to methanol toxicity and, consequently, extrapolation of results from methanol studies with non-primates to human beings is not possible (Tephley, 1991). In studies with monkeys, however, the administration of methanol produced clinical effects (depression, anorexia, weakness, vomiting, hyperpnea, tachypnea, and dilated unresponsive pupils), morphological effects (edema of optic disc and optic nerve), and biochemical findings (acidosis, decrease in concentrations of blood $HCO_3$) consistent with those described in cases of human poisoning (McMartin *et al.,* 1975; Hayreh *et al.,* 1977; Clay *et al.,* 1975; Baumbach *et al.,* 1977). Metabolic acidosis and ocular toxicity are produced by accumulation of formate, a metabolic intermediate in methanol catabolism (Clay *et al.,* 1975; Tephly, 1977). Ocular toxicity results from formate accumulation and is independent of the development of metabolic acidosis (Hayreh *et al.,* 1977). In primates and monkeys, the primary system for formate metabolism is the folate-dependent pathway which converts formate to $CO_2$ and tetrahydrofolate. Inhibition of this system by feeding folate-deficient diets to rats, or by exposure of rats and monkeys to nitrous oxide (which inhibits activity of methionine synthetase, the enzyme that catalyzes the conversion of 5-methyl tetrahydrofolate to tetrahydrofolate), decreases hepatic concentrations of tetrahydrofolate and decreases rates of formate oxidation (Eells *et al.,* 1981; 1982; 1983). Additionally, activity of 10-formyl tetrahydrofolate dehydrogenase, the enzyme that catalyzes the conversion of 10-formyl tetrahydrofolate (which results from the metabolism of formate and tetrahydrofolate) to $CO_2$ and tetrahydrofolate, is much lower in human and monkey liver than in rat liver (Johlin *et al.,* 1987). Therefore, the insensitivity of rodents to methanol and, consequently, formate toxicity results from high levels of hepatic tetrahydrofolate and rapid metabolism of 10-formyl tetrahydrofolate to $CO_2$ and tetrahydrofolate. In future studies of formic acid/formate toxicity, the use of more susceptible species such as nonhuman primates or swine should be considered.

In conclusion, buffered solutions of formic acid were not mutagenic in *Salmonella*. Inhalation exposures to formic acid for 2 and 13 weeks in F344/N rats and $B6C3F_1$ mice produced minimal systemic toxic effects. At 13 weeks, hematologic and biochemical changes were mild and consistent with hemoconcentration. Gross and microscopic changes were confined to the upper respiratory tract and were consistent with effects produced by irritant chemicals administered by inhalation exposure. Effects on the respiratory and olfactory epithelium at 13 weeks consisted of squamous metaplasia (minimal, rats) and degeneration (minimal, rats and mice), respectively. Based on the findings in the 13-week studies, the no-observed-adverse-effect-level (NOAEL) for microscopic lesions in rats and mice was 64 ppm, but a lower NOAEL (32 ppm) was determined based on respiratory lesions present at the end of the 2-week study. The lack of systemic effects in either the 2- or 13-week studies may be related to the ability of rodents to rapidly metabolize formate to $CO_2$. Because humans metabolize formate less readily than rodents and are significantly more sensitive to its toxicity, caution should be used in considering the results of these studies in determining potential human risks associated with systemic exposure to formic acid.

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